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THE STERILIZATION OF AMERICAN FOULBROOD COMBS

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CONTENTS

INTRODUCTION

A new era in the treatment and control of American foulbrood has been opened by the use of disinfectants such as an alcohol-formalin solution for the sterilization of combs infected with this disease of the brood of bees. Widespread interest has been aroused throughout the beekeeping industry by the apparent success of this method of treatment, which eliminates to a great extent the large losses previously caused by the necessary destruction of all combs infected with American foulbrood.

Numerous attempts have in the past been made to use formaldehyde gas as a disinfectant, but it failed to sterilize infected combs completely except in a few carefully controlled cases, so that eventually the use of this material for disinfecting combs was entirely abandoned. White (*25*, *26*)[1] found that the gas penetrated the combs slowly, even when used in a tightly sealed jar, and that as usually applied in the apiary it was insufficient to disinfect the combs completely. More recently Maassen and Borchert (*18*) and Borchert (*1*), experimenting with formaldehyde gas generated from

[1] Reference is made by number (italics) to "Literature cited," p. 27.

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solid paraformaldehyde, came to a similar conclusion. The great difficulty always was found to be the holding of the gas in contact with the combs, without leakage to the outside, for a sufficiently long time to allow for this slow penetration and the complete sterilization of all the spores of *Bacillus larvae.*

Late in 1922 the apparently successful use of a new liquid disinfectant for treating infected combs was announced by J. C. Hutzelman, of Glendale, Ohio (*11*). The disinfectant is composed primarily of 20 parts of formalin solution to 80 parts of specially denatured alcohol. The infected combs are placed in a tank and completely immersed in this liquid for 48 hours. At the end of that period the combs are removed and as much as possible of the excess liquid is removed from them, an extractor being used when available. They are then allowed to dry, until no odor of formaldehyde remains, before the combs are returned to healthy colonies. The use of alcohol as the carrier for the formaldehyde, which is the active sterilizing agent in the solution, is based, according to Hutzelman, upon two properties. Since alcohol is a liquid of low surface tension the solution is supposed to enter readily and permeate thoroughly all empty spaces in the combs. Further, it is claimed that this alcoholic solution, because of its solvent action, penetrates the wax and propolis, as well as cells filled with pollen, all of which may contain spores of *Bacillus larvae.* Hutzelman is of the opinion that the sterilization of all of these is necessary for the successful disinfection of infected combs. He has also stated that while all sealed honey cells must be opened, sealed brood cappings need not be removed if the combs are treated for 48 hours, as the brood cappings are porous and are penetrated by the alcoholic liquid. Much credit is due Doctor Hutzelman for thus being the first to demonstrate the practical possibility of holding the formaldehyde in more or less direct contact with the diseased material in combs infected with American foulbrood for a sufficiently long period to kill the spores of *B. larvae.*

In June, 1924, Dan H. Jones (*14*), of Ontario, Canada, reported the results of some cultural experiments with various disinfectants, including the alcohol-formalin and also water-formalin solutions. He states that the alcohol-formalin solution

> is effective in killing the spores of *Bacillus larvae* in 24 hours in foulbrood affected combs when it comes in contact with the larval scales in open cells. In case of capped cells, however, it would appear that 48 hours' immersion is essential.

Concerning various mixtures of water and formalin he says:

> Formalin diluted with water in as high a dilution as 15 per cent formalin to 85 per cent of water is effective in destroying in 24 hours the spores of *Bacillus larvae* as they occur in larval scales in open cells. Although in our experiment a 48-hour immersion in the formalin and water mixtures gave us a hundred per cent sterile results in the cultures made from capped cells, we can scarcely expect that such would always be the case, as the porosity of the cell caps varies considerably.

In a recent article Jones (*15*) gives a further report of investigations with water-formalin solution, as indicated by the following table from his paper:

Disinfectant	Combs immersed 24 hours						Combs immersed 48 hours					
	Cultures from capped cells			Cultures from uncapped cells			Cultures from capped cells			Cultures from uncapped cells		
	1	2	3	1	2	3	1	2	3	1	2	3
Formalin 100	0	0	0	0	0	0	0	0	0	0	0	0
Formalin 50, water 50	0	0	0	0	0	0	0	0	0	0	0	0
Formalin 25, water 75	0	0	0	0	0	0	0	0	0	0	0	0
Formalin 20, water 80	0	0	+	0	0	0	0	0	0	0	0	0
Formalin 15, water 85	0	0	+	0	0	0	0	0	0	0	0	0
Formalin 10, water 90	0	+	+	0	0	0	0	0	0	0	0	0
Formalin 50, alcohol 50	0	0	0	0	0	0	0	0	0	0	0	0
Formalin 20, alcohol 80	0	0	0	0	0	0	0	0	0	0	0	0
Hutzelman's solution	0	0	+	0	0	0	0	0	0	0	0	0

He further says:

It will be seen that in the case of uncapped cells, after twenty-four hours' immersion in all dilutions of formalin used, the spores of *B. larvae* were killed. In case of the capped cells, however, a few of the spores were not killed in this length of time either in the water dilutions or in Hutzelman's solution.

After forty-eight hours' immersion, however, all spores were killed in capped cells as well as in uncapped cells. Thus, in these experiments, the water dilutions of formalin proved to be as effective as the alcohol solutions in destroying the spores of *B. larvae* as they occur in the scales of the infected brood combs.

Although this method has been in use for only about two years, one of the bee journals has published several reports concerning the successful use of the alcohol-formalin solution, describing in all the treatment of several thousand combs. One of the most notable cases is that of O. E. Barber, of Ohio, reported by G. S. Demuth (*8*), in which some 6,000 combs were treated during the summer of 1923, apparently without recurrence of disease. There have been other similar cases, all indicating that this solution is satisfactory. On the other hand, during the latter part of the summer of 1924, J. L. Byer, of Ontario, Canada, who had treated 400 infected brood combs and 1,100 super combs (*2*), found several cases of recurrence of disease (*3*) in colonies made from package bees placed on these treated combs. Some of these combs were sent to Doctor Jones, but cultures made from them failed to give growth (*4*). Two similar cases of recurrence of disease after the use of an alcohol-formalin solution have also come to the attention of the Bee Culture Laboratory, through correspondence. So far as can be learned, all precautions were taken in these cases, and no adequate explanation can be given for the recurrence of disease other than that for some reason the disinfection could not have been complete.

The alcohol-formalin treatment of foulbrood combs received considerable discussion at the annual convention of the Ontario Beekeepers' Association in 1925, as reported by Byer (*5*) in one of the bee journals. He says:

While reports in the main were favorable, yet some outstanding failures were reported, and the department at Guelph is not yet prepared to advise the treatment of badly diseased combs from the brood chamber. They do, however, recommend treating all super combs from diseased colonies, as every test made with such combs failed to produce any disease. From the address of

Professor Jones (*15*) we learned that in every case water used with the formalin gave just as good results as when alcohol was used. The assistant apiarist, Mr. Jarvis, also substantiated these conclusions.

In a recent article G. L. Jarvis (*13*), of the Ontario Agricultural College, Guelph, Ontario, describes results of experiments in the apiary in collaboration with Doctor Jones concerning the effectiveness of water-formalin and alcohol-formalin solutions. Colonies made with 2-pound packages of bees were used in which to test combs treated in various ways. He says:

> From the results of these experiments and laboratory tests we have arrived at these conclusions: First, that both the formalin water and alcohol formalin solution will kill all germs causing American foulbrood in open cells. Second, that there is still a doubt as to the effectiveness of both solutions in the case of capped cells.
>
> With this in mind, we are ready to advocate the use of the formalin water solution for sterilizing super combs from diseased colonies after the honey has been extracted. The strength of the solution must not be less than 15 per cent formalin to 85 per cent water and the combs immersed for at least 12 hours. The uncapping must be well done; that is, there must be practically no sealed honey in the combs. The combs should be held, if possible, until the following spring before being given back to the bees.
>
> Although in our experiments so far we have had no reappearance of disease in treated brood combs, except in check colonies, some other beekeepers who have tried the alcohol-formalin treatment have not been so successful. However, the super comb is the doubtful comb, and we believe it is well worth while to know of a cheap solution, such as the formalin-water, which will eliminate this doubt. Again, if all combs from a diseased colony were melted or burned there would be approximately three super combs destroyed to every brood comb, and the super combs would likely average a better quality than those in the brood chamber.

G. H. Vansell, in California (*23*), recently has reported promising experiments in sterilizing foulbrood combs, in which he used various mixtures of formalin in soapy waters. The results were found encouraging as to sterility and cost. No details are given concerning methods or results.

Since the announcement of this new method of treating combs many beekeepers, as well as the investigators cited above, have been stimulated to experiment for themselves. In 1924, 33 samples of treated comb were submitted by beekeepers to the Bee Culture Laboratory for cultural examinations as to the sterilizing efficiency of the various solutions used. In a majority of cases the disinfectant used was not described. In about one-third of the cases some variation of the alcohol-formalin solution as devised by the person sending the sample was used. Three of these cases, however, were specifically said to have been treated with the commercial alcohol-formalin solution. Of the 33 samples, 10 gave cultures showing growth of *Bacillus larvae* from both open and sealed cells. Fourteen of the 33 samples, including 2 samples that had been treated in the commercial solution, gave cultures showing no growth from open cells but good vegetative growth of *B. larvae* from many of the sealed cells. Nine of the 33 samples, only 1 of which was known to have been treated with the commercial solution, gave cultures showing no growth from either open or sealed cells, thereby indicating complete sterilization.

It would, therefore, seem probable that, although the method of treating infected brood combs with an alcohol-formalin solution is a step in advance in the control of American foulbrood, there apparently

is room for improvement which will eliminate the danger of occasional cases of failure. Because of the widespread interest in this subject, preliminary work was started early in 1924 at the Bee Culture Laboratory with the purpose of making an exhaustive bacteriological study of the efficiency of various disinfectants, including the commercial alcohol-formalin solution as well as water-formalin solutions. It was hoped that the results of the investigation by laboratory methods would form a basis for practical work in the apiary. As the work has developed, numerous difficulties have been encountered which indicate that the problem of the perfect sterilization of American foulbrood combs is neither simple nor as yet fully solved.

The results given herein are of a preliminary nature, the data being in some cases incomplete; but they are given for what they seem to indicate. Since these investigations were started Doctor Hutzelman has taken out a patent (*12*) on the solution devised by him, issued October 14, 1924. In the light of the issuance of this patent it seems advisable to state that the United States Department of Agriculture can assume no responsibility for the use of any of the solutions or processes described and discussed in this paper if they in any way infringe the patent.

METHODS

DISEASED MATERIAL FOR TESTS

In devising methods for testing the efficiency of various disinfectant solutions, procedures were adopted corresponding as closely as possible, on a reduced scale, to the actual practice of beekeepers. Combs affected with American foulbrood, containing as many scales as possible both in sealed cells and in open cells, were obtained from various sources. From the brood areas of these combs test pieces were cut approximately of the standard size of 1½ by 2½ inches. Glass specimen jars of about 160 cubic centimeters capacity, with fitted glass covers, were used to hold the test solutions and pieces of comb treated. A standard volume of liquid of 100 cubic centimeters was used throughout the tests, the proportion of liquid to comb being approximately that in the the regular 10-frame tanks used by beekeepers for disinfecting combs. Loss of liquid after each consecutive comb had been removed was made up to 100 cubic centimeters with more solution before a new piece was immersed. Several pieces of comb were passed through each lot of solution consecutively, in keeping with the actual apiary practice of treating many combs in the same solution. The piece of comb to be tested was placed in the empty jar and fastened down with a wire spring to prevent its floating. The solution was poured into the jar slowly to permit the liquid more readily to enter the open cells. In accordance with the most approved apiary practice, there was no shaking of combs to aid in removal of air bubbles. The test combs were then allowed to soak for 24 or 48 hours, as the case might be. No immersions of less than 24 hours were tried in this series of experiments.

Upon removal of the pieces of comb from the disinfectant, as much as possible of the excess liquid remaining in the cells and on the surface of the comb was removed by vigorous shaking. Each

piece was then inclosed in a piece of filter paper to protect it from dust and was allowed to dry at the ordinary room temperature of the laboratory until no odor of formalin could be detected.

CULTURES

Yeast-extract egg-yolk agar medium, as described in a recent paper by the writer (*21, p. 136*) was used in the form of slants for the purpose of making the cultural tests of the scales treated by the various solutions. In order that there should be a sufficient amount of water of condensation at the base of the slants, the necessary number of tubes of fresh medium for each group of combs to be tested were made up by the addition of sterile egg yolk to tubes of the base medium. With each new lot of medium control cultures were made from untreated scales of American foulbrood.

The removal of scales or other diseased remains from the cells of the treated combs was accomplished by means of a lancet-shaped dissecting needle which had just been sterilized in a flame and allowed to cool. The scale (or other remains) was then carefully placed in the water of condensation of the tube of culture medium, one scale or material from one cell to a tube of medium. Cappings from sealed brood were removed with the hot needle, and after resterilizing the needle the scale contained in one cell was removed and placed in a culture tube. After the diseased material had been allowed to soak in the condensation water of the tube for about an hour, or longer if necessary, until the dried scale had softened, it was macerated and spread over the surface of the slant by means of a stiff platinum loop.

Cultures were at first made of material from one open cell and one sealed cell from each comb treated, on the assumption that for a given solution the sterilization would be uniform for a definite period of time, but it soon became evident that there was a variation in the rapidity with which the various solutions penetrated the brood cappings. At least five cultures were therefore made from open cells and five from sealed cells in each piece of comb, amounting to approximately 3 to 5 per cent of all cells in a piece of comb of the size used in these experiments. The percentage cultured of the scales actually present is really much higher than this, since a piece of comb of that size seldom has a scale in every cell.

After incubation for 48 hours at 37° C., cover-glass smears were made from the material on the surface of the slants. A large loopful of a mixture of material from different parts of the slant in a drop of distilled water was used in making a good-sized smear on the cover glass. After drying the cover glass in the air and passing it quickly through a flame three times the smears were stained for about 20 seconds with Ziehl-Neelson carbol-fuchsin diluted with an equal quantity of distilled water. The cover glasses were then carefully washed in water, dried, mounted with Canada balsam, and examined under the microscope. At least 20 to 30 fields were examined to determine whether there had been any germination of spores of *Bacillus larvae* or vegetative growth not visible on the slant. When no germination of spores was observed, as a rule only one examination of the culture was made at the end of 48 hours' incubation, since if there are any spores in a condition to germinate

they will do so within that period. When a few spores were found to have germinated, but without evidence of any vegetative growth, a subculture was generally made, using a generous quantity of material from the surface of the original slant. When good vegetative growth was observed it was so recorded as positive growth. In some instances when there was doubt regarding a culture several more cells of the same kind were cultured from the same piece of comb.

SOLUTIONS TESTED

In the earlier part of this investigation an attempt was made to find a substitute for alcohol as a carrier for the disinfectant, because of the difficulty of purchasing pure grain alcohol and the comparatively high cost of the commercial alcohol-formalin solution. Since certain substances are used in various insecticide sprays to increase the wetting and spreading powers of the spray solutions (*6*, *19*), it was suggested that if a liquid could be found which would spread easily over the surface of the wax comb and diseased remains, formalin added to such a liquid would be carried by it and brought into contact with those remains; and that it would penetrate sufficiently to kill the spores, even though the cells, particularly sealed cells, might not be completely filled with the liquid. This property in insect sprays is often obtained by the addition of soaps of various kinds to the solutions. The addition of soap also tends to lower the surface tension of a water solution. Dilute solutions of various types of soaps, both soft and hard, were used to form the carrier for the 20 per cent of formalin. By experimentation with dry pieces of comb it was found that only a comparatively small quantity of soap can be used in a solution, as too much causes the liquid to become viscous and jelly-like, inhibiting its entrance into the cells. In the tests with soap solutions varying quantities of half-normal sodium oleate soap, cottonseed-oil soap, and a 10 per cent solution of one of the common hard toilet soaps were used in making up the solutions for the disinfection of diseased combs. From 0.5 to 2 cubic centimeters of each of the various soaps was used per 100 cubic centimeters of a 20 per cent solution of formalin in water; if much more than 2 cubic centimeters was used the solution became too viscous.

In a recent article King (*16*) describes experiments in sterilizing American foulbrood combs by using a disinfectant solution consisting of the 20 per cent water-formalin solution, with sufficient soap to form suds (about 1 pound to 5 gallons of solution), the addition of the soap causing the solution to enter the cells more readily. After 48 hours' immersion, followed by extraction and a short drying period, the combs were given to a healthy colony. Only one colony was used in the experiment, but King reports that no disease appeared in it when observed carefully at intervals for seven weeks after it had been given the treated combs.

Among substances similar in physical properties to ethyl alcohol, a commercial methyl-ethyl ketone solution, acetone, and iso-propyl alcohol were tried as carriers for formalin, but acetone and iso-propyl alcohol were too expensive for practical use.

The use of hydrochloric acid in the sterilization of imported hides, as a preventive of anthrax (*20*), a disease caused by a spore-

forming organism similar in nature to *Bacillus larvae*, suggested its use in the treatment of American foulbrood. Various dilute solutions of iodine were suggested, since iodine is successfully used in the sterilization of drinking water. A solution of formalin in water, with the addition of acetic acid to increase its penetrating power, is often used for fixating histological preparations; for the treatment of American foulbrood a similar solution was accordingly suggested.

A number of tests were made to compare the germicidal efficiency of the commercial alcohol-formalin solution, now frequently used by beekeepers, with that of the various other solutions tried, and in particular with the 20 per cent solution of formalin in water. Krönig and Paul (*17*) have shown that increasing quantities of either ethyl or methyl alcohol added to a solution of formalin in water progressively decreases the germicidal efficiency of the solution. Tests were therefore made with various dilutions of denatured alcohol as the carrier for the 20 per cent solution of formalin. Formula No. 1 (*22, p. 100*) for specially denatured alcohol was used as a basis for these dilutions. Commercial alcohol-formalin solution was diluted in a similar manner by the addition of varying quantities of a 20 per cent solution of formalin in water. These tests were made to determine whether the presence of a greater proportion of water mixed with the alcohol changes the germicidal efficiency of the solution.

In this preliminary work no measurements were made of surface tension and other physical properties, but the bacteriological results were used as a criterion of the relative efficiency of the various disinfectant solutions tested.

OBSERVATIONS

SOAP SOLUTIONS

In the use of soap-formalin solutions it was found immaterial whether soft or hard soap is used. The greatest difficulty with the soap solutions, aside from the fact that they do not seem to penetrate sealed cells uniformly, which will be discussed later, is a rapid change in reaction from alkalinity to marked acidity during the period of immersion of one piece of comb. The acid reaction causes the soap gradually to precipitate, so that the solution is soon little better than ordinary water-formalin solution. Even the addition of sufficient normal sodium-hydroxide solution to bring the reaction back to alkaline after the comb is removed fails to remedy this fault, as the precipitated soap does not return into solution on the addition of the sodium hydroxide. The prolonged action of soap and alkali on the wax of the comb during an immersion of 48 hours causes more softening of the cell walls than is desirable, and makes the combs quite fragile. Since some of the sealed cells in practically every series of combs tested failed to be sterilized, as indicated from the cultures made (Table 1), this solution was dropped from consideration for use with combs having sealed brood.

In the case of the sodium-oleate-formalin solutions, 59 open and 59 sealed cells were cultured in 10 series, varying in length (Table

1). Cultures from 54 open cells showed no growth of *Bacillus larvae*, whereas 5 culture tubes were contaminated. Cultures from 45 sealed cells showed no growth of *B. larvae*, 2 culture tubes were contaminated, 11 showed good growth of *B. larvae*, and 1 showed only a few spores germinated, or 12 positive cultures in all. In brief, of the sealed cells 20.3 per cent were not sterilized.

TABLE 1.—*Cultural results of various tests with samples of comb treated 48 hours in water-soap-formalin solution*

Kind of soap	Composition of solution: Soap	Water	Formalin solution	Normal sodium hydroxide	Number of consecutive pieces of comb immersed in same lot of solution	Open cells: Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	Cultures contaminated	First comb in series containing open cells showing growth of *B. larvae* in cultures	Sealed cells: Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	Cultures contaminated	First comb in series containing sealed cells showing growth of *B. larvae* in cultures
	C. c.	*C. c.*	*C. c.*	*C. c.*											
Half-normal sodium oleate.	0.5	80	20	0	4	4	0	3	1	.....	4	0	4	0	
	0.5	80	20	0	4	4	0	3	1	.....	4	0	2	2	
	1	80	20	0	5	5	0	5	0	.....	5	1	4	0	Third.
	1	80	20	0	5	5	0	4	1	.....	5	2	3	0	Do.
	1	80	20	1	6	6	0	6	0	.....	6	0	6	0	
	1	75	20	5	6	6	0	6	0	.....	6	0	6	0	
	1	80	20	1	9	9	0	8	1	.....	9	[1] 1	8	0	Second
	1	80	20	1	5	5	0	5	0	.....	5	2	3	0	First.
	2	80	20	2	9	9	0	8	1	.....	9	2	7	0	Sixth.
	[2] 2	80	20	1	6	6	0	6	0	.....	6	4	2	0	First.
10 per cent solution of hard toilet soap.	1	80	20	1	9	9	0	9	0	.....	9	1	8	0	
	2	80	20	2	9	9	0	9	0	.....	9	[3] 2	7	0	Third.
	1	80	20	1	2	2	0	2	0	.....	2	[1] 1	1	0	Second.
	1	80	20	1	4	4	0	3	1	.....	4	[3] 2	1	1	First.
	1	80	20	1	7	[4] 7	0	7	0	.....	7	4	3	0	Second.
	2	75	25	0	4	4	0	4	0	.....	4	3	1	0	First.
	2	75	25	0	4	4	0	4	0	.....	4	3	1	0	Second.
	2	80	20	2	6	[5] 6	0	6	0	.....	[5] 6	0	6	0	
	2	80	20	2	6	[5] 6	0	6	0	.....	[5] 6	0	6	0	
	0.5	90	10	0	4	4	0	4	0	.....	4	1	3	0	First.
	1	90	10	0	4	4	0	4	0	.....	4	[1] 1	3	0	Fourth.
	0.5	85	15	0	4	4	[3] 2	2	0	First.	4	2	2	0	First.

[1] Few spores germinated.
[2] Cottonseed-oil soap.
[3] One showed few spores germinated.
[4] Used entire brood combs in tank.
[5] Sealed cells with cappings perforated compared with open cells.

In the case of the solutions of hard toilet soap in 20 per cent formalin, 39 open and 39 sealed cells were cultured. Cultures from 38 open cells showed no growth, and the culture from 1 was contaminated. From the sealed cells, 22 cultures showed no growth, 1 was contaminated, 13 showed good growths of *Bacillus larvae*, and in 3 only a few spores had germinated; in all, there were 16 positive cultures, showing that 41 per cent of the sealed cells were not sterilized. The variation in the quantity of soap added to the solution seemed to have little effect on the variable penetration and sterilization of the scales in sealed cells, but the hard-soap solutions appeared somewhat less efficient than the solutions of soft soap.

Three short series (see bottom of Table 1) were tried, using smaller quantities of formalin, but these solutions proved inefficient as germicides in open as well as in sealed cells.

LIQUIDS OF LOW SURFACE TENSION OTHER THAN ETHYL ALCOHOL

The results of tests with these liquids are presented in Table 2. Acetone gave indications of being a rather more efficient carrier than those previously used, both in the proportions of 80 parts acetone to 20 parts formalin, and even when diluted with water in the proportions of 50 parts acetone to 30 parts water and 20 parts formalin. Two series, one of 4 open and one of 4 sealed consecutive combs, and one series of 9 sealed combs, showed no cells giving growth of *Bacillus larvae*, while each of three series of sealed combs showed only 1 cell giving growth. Two short series were tried with the use of smaller proportions of formalin, 15 parts and 10 parts, respectively, per 100 parts. These proportions proved to be inefficient as germicides in both open and sealed cells. In the light of later work, it seems possible that if more cells from each comb had been examined a few more positive cultures from sealed cells might have been found; but in comparison with other solutions tested under the same procedure of cultural examination, acetone gave about the best results as a carrier. The comparatively high cost of acetone eliminates it as a substance that can be used economically in apiary practice.

The ketone solution previously mentioned was mixed with formalin in the proportions of 80 parts ketone to 20 parts formalin. This solution, as may be seen from the table, sterilized the open cells, but was unsatisfactory in its action on scales in sealed cells.

TABLE 2.—*Cultural results of various tests with samples of comb treated for 48 hours in liquids of low surface tension, other than ethyl alcohol*

Liquid used	Composition of solution			Number of consecutive pieces of comb immersed in same lot of solution	Open cells				First comb in series containing open cells showing growth of *B. larvae* in cultures	Sealed cells				First comb in series containing sealed cells showing growth of *B. larvae* in cultures
	Liquid used	Water	Formalin solution		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	Cultures contaminated		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	Cultures contaminated	
	C.c.	*C.c.*	*C.c.*											
Acetone	80	0	20	4	4	0	4	0	----------	4	0	4	0	
	80	0	20	10	10	0	10	0	----------	10	1	9	0	Tenth.
	80	0	20	10	10	0	10	0	----------	10	1	9	0	Do.
	50	30	20	9	9	0	9	0	----------	9	0	9	0	
	50	30	20	9	9	0	9	0	----------	9	[1] 1	8	0	Fifth.
	40	40	20	4	4	0	3	1	----------	4	0	4	0	
	85	0	15	3	3	[1] 1	2	0	Second	3	1	2	0	Second.
	90	0	10	3	3	[1] 1	2	0	Third	3	1	2	0	Third.
Methyl-ethyl ketone	80	0	20	5	5	0	5	0	----------	5	[2] 4	1	0	First.
	80	0	20	5	5	0	4	1	----------	5	[3] 3	1	1	Second.
	80	0	20	5	5	0	3	2	----------	5	1	3	1	Do.
Iso-propyl alcohol	50	30	20	7	7	0	7	0	----------	7	[1] 1	6	0	Fifth.
	50	30	20	7	7	0	7	0	----------	7	0	7	0	

[1] Few spores germinated. [2] Three showed few spores germinated. [3] Two showed few spores germinated.

Iso-propyl alcohol, another liquid manufactured on a commercial scale resembling ethyl alcohol in its physical properties, but too

expensive for practical use, was also tested. Only four series, each of seven cells, were tried with this carrier for purposes of comparison, two of open and two of sealed cells, the proportions used being 50 parts of iso-propyl alcohol, 30 parts of water, and 20 parts of formalin. Of all the 28 cells cultured only one sealed cell was found to contain viable *Bacillus larvae*. This solution is therefore in about the same class as that of acetone.

MISCELLANEOUS SOLUTIONS

The results of experiments with these solutions are presented in Table 3. The use of iodine solutions proved entirely impracticable for sterilizing infected combs. In dilutions of 1 to 50,000 in water and even 1 to 500, with an immersion of 48 hours, no germicidal action on the spores from diseased material in either open or sealed cells could be demonstrated. A solution of 1 to 20 iodine killed all the spores, both in open and sealed cells, but the comb was attacked to such an extent as to make it too soft for further use. The probable reason for the lack of germicidal action on the part of iodine is that this substance combines readily with the fatty-acid constituents

TABLE 3.—*Cultural results of various tests with samples of comb treated for 24 hours in various solutions*

Composition of solution	Duration of immersion	Number of consecutive pieces of comb immersed in same lot of solution	Open cells					Sealed cells				
			Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	Cultures contaminated	First comb in series containing open cells showing growth of *B. larvae* in cultures	Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	Cultures contaminated	First comb in series containing sealed cells showing growth of *B. larvae* in cultures
	Hours											
Iodine, 1–50,000 water	48	2	2	2	0	0	First	2	2	0	0	First.
Iodine, 1–500 water	48	1	1	1	0	0	do	1	1	0	0	Do.
Iodine, 1–20 water	48	2	2	0	2	0		2	0	2	0	
Denatured alcohol (50 per cent), 93 c. c., hydrochloric acid, 5 c. c., formalin, 2 c. c.	24	4	4	0	4	0		4	4	0	0	Do.
Denatured alcohol, 90 c. c., hydrochloric acid, 5 c. c., formalin, 5 c. c.	24	4	4	1	3	0	Third	4	4	0	0	Do.
Denatured alcohol (50 per cent), 93 c. c., hydrochloric acid, 5 c. c., formalin, 2 c. c.	48	3	3	0	3	0		3	3	0	0	Do.
Denatured alcohol, 90 c. c., hydrochloric acid, 5 c. c., formalin, 5 c. c.	48	3	3	1	2	0	First	3	3	0	0	Do.
Acetic acid, 10 c. c., water, 70 c. c., formalin, 20 c. c.	48	8	8	0	8	0		8	3	5	0	Fourth.
Do	48	8	8	0	8	0		8	3	5	0	Do
Acetic acid, 5 c. c., water, 75 c. c., formalin, 20 c. c.	24	7	7	0	7	0		7	3	4	0	Do.
Do	48	3	3	0	3	0		3	2	1	0	Second.
Denatured alcohol, 80 c. c., formalin, 20 c. c., glycerin, 2 c. c.	24	4	4	0	4	0		4	4	0	0	First.
Do	48	5	5	0	5	0		5	1	4	0	Third.
1 per cent solution of gelatine, 80 c. c., formalin, 20 c. c.	48	3	3	0	3	0		3	[1] 2	1	0	Second.
Do	48	3	3	0	3	0		3	[2] 1	2	0	Do.
10 per cent ketone solution added to 95 per cent alcohol, 80 c. c., formalin, 20 c. c.	48	7	7	0	6	1		7	2	5	0	Do.

[1] One showed few spores germinated.

[2] Few spores germinated.

of the wax, as well as with the fatty residue in the diseased remains of the brood, thereby nullifying the action of the iodine as a germicide.

The use of denatured alcohol in various dilutions containing 5 per cent concentrated hydrochloric acid, and a small quantity of formalin to prevent deleterious action of the acid (*10*) on the supporting wires of the frame, gave unsatisfactory results with immersions of 24 and of 48 hours. Each of the sealed cells tested gave a good growth of *Bacillus larvae*; in two cases growth was obtained from open cells, although some spores apparently were killed. This type of solution was not tested further.

Varying quantities of acetic acid added to a 20 per cent solution of formalin in water contributed nothing to the germicidal or penetrating action of the solution so far as sealed cells were concerned, as several such cells in each series tested gave each a good growth of *Bacillus larvae*. These results are very similar to those obtained with plain water-formalin solution, which will be considered later.

Several other miscellaneous solutions were tried whose composition is indicated in the table, all containing 20 per cent of formalin, but varying in the composition of the carrier. All proved unsatisfactory when used with sealed cells, and will not be discussed further.

SOLUTIONS WITH DILUTED ALCOHOL

A few dilutions of denatured alcohol, as well as dilutions of alcohol-formalin solution, were made, varying the alcohol content from about 30 per cent to over 60 per cent, as indicated in Table 4, the 20 per cent formalin, however, being kept constant in the mixtures with denatured alcohol. Tests were made of combs treated for 24 hours and for 48 hours. In every case no growth was obtained from open cells, and, as would be expected, there were fewer positive cultures from sealed cells in combs treated 48 hours than from those treated 24 hours, although several sealed cells showed no growth, even in the 24-hour series. In each of the 48-hour series there was at least one sealed cell from which was obtained a growth of *Bacillus larvae*, but, with the few observations made, no significant differences could be found between the few dilutions tested of alcohol or alcohol-formalin solution. Further work is necessary to demonstrate whether results comparable with those of Krönig and Paul can be obtained.

For purposes of comparison, as a preliminary test, three series of six samples each from diseased combs were treated with a solution containing no alcohol, composed of formalin 20 parts and water 80 parts (Table 9). No significant difference could be seen between these results and those obtained with the various alcoholic solutions. No open cells treated with this solution gave cultures showing growth, but 8 sealed cells, 1 or more in each series, gave cultures showing growth of *Bacillus larvae*, the growths in 3 of the 8 cultures being noted as "few spores germinated."

TABLE 4.—*Cultural results of various tests with samples of comb treated for 24 hours and for 48 hours in various dilutions of alcohol-formalin and commercial alcohol-formalin solutions*

Composition of solutions	Duration of immersion	Number of consecutive pieces of comb immersed in same lot of solution	Open cells			First comb in series containing open cells showing growth of *B. larvae* in cultures	Sealed cells			First comb in series containing sealed cells showing growth of *B. larvae* in cultures
			Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	
	Hrs.									
Commercial alcohol-formalin solution, 50 c. c., 20 per cent formalin in water, 50 c. c.	24	4	4	0	4	----------	4	1	3	First.
Do	48	7	7	0	7	----------	7	2	5	Sixth.
Commercial alcohol-formalin solution, 40 c. c., 20 per cent formalin in water, 60 c. c.	24	7	7	0	7	----------	7	[1] 5	2	Third.
Do	48	9	9	0	9	----------	9	1	8	Seventh.
Denatured alcohol (62.5 per cent), 80 c. c., formalin, 20 c. c	24	7	7	0	7	----------	7	[1] 3	4	Third.
Do	48	5	5	0	5	----------	5	[2] 1	4	Fourth.
Denatured alcohol (50 per cent), 80 c. c., formalin, 20 c. c	24	7	7	0	7	----------	7	[1] 2	5	Third.
Do	48	5	5	0	5	----------	5	1	4	Fourth.
Denatured alcohol (40 per cent), 80 c. c., formalin, 20 c. c	24	7	7	0	7	----------	7	3	4	Fourth.
Do	48	7	7	0	7	----------	7	2	5	Sixth.
Denatured alcohol (50 per cent), 75 c. c., formalin, 20 c. c., glycerin, 5 c. c	24	4	4	0	4	----------	4	2	2	First.
Do	48	5	5	0	5	----------	5	[1] 2	3	Fourth.

[1] One showed few spores germinated. [2] Few spores germinated.

COMMERCIAL ALCOHOL-FORMALIN SOLUTION

Ten series of combs were treated with the commercial alcohol-formalin solution at various times. From these combs 61 open and 61 sealed sells were cultured. It was intended to use these series as controls for comparison with other solutions, since published reports of the success obtained with this commercial solution in apiary practice indicated that negative cultural results would be obtained. As may be seen in Table 7, however, in the case of every one of the 10 preliminary 48-hour series 1 or more sealed cells, 22 in all, or 36 per cent, were found to give a growth of *Bacillus larvae*. In 3 cases of the 22 the growths were noted as "few spores germinated." No growth was obtained from the 61 open cells cultured. At first it was thought that the solution might have deteriorated; fresh solution was obtained and tested, with practically the same results. A chemical analysis of the actual formaldehyde content of the fresh solution, and a similar analysis of solution through which some 500 combs had been passed, showed an actual increase with use in the percentage of formaldehyde. This increase was without doubt due to the more rapid evaporation of the alcohol than of the formaldehyde content. These results seem to indicate that there must be a variation in the permeability of cappings, the decrease in permeability slowing up or even preventing the penetration of disinfectants to the scales and spores.

PERMEABILITY OF CAPPINGS

A simple experiment was undertaken in an effort to learn whether the permeability of the cappings is variable. Such a variation might account for the fact that sealed cells are not always sterilized, and for the variation in the number of cultures of *Bacillus larvae* from sealed cells in different combs.

If cappings are carefully removed from a piece of comb and examined under the microscope, it is seen that their structure is apparently very variable. The cappings are composed of criss-crossing cocoon fibers, pollen grains, and granules of wax, and consequently vary in structure. Cappings from brood cells of different ages are found to vary greatly in thickness. Freshly sealed cappings are much thicker and more opaque than cappings from the cells of more nearly matured pupæ, the latter often being gnawed by adult worker bees.

A simple piece of apparatus was devised to test the variability in porosity of cappings. A piece of glass tubing slightly smaller in outside diameter than the inside diameter of worker cells was drawn to a fine capillary tube and broken off, making a capillary opening on the end of a 6-inch tube. Numerous cappings were then removed from various samples of comb, both diseased and healthy, with a sharp scalpel, and cut so as to leave on the capping a rim of cell wall about one-sixteenth to one-eighth-inch wide, care being taken not to rupture the capping. These cappings were then sealed on the larger end of the glass tube with liquefied beeswax. The end of the tube covered by the capping was then submerged 3 centimeters below the surface of the chosen disinfectant, so as to have a uniform upward pressure on all the cappings successively tested. The rise of the liquid in the tube was measured at the end of a five-minute period unless the liquid was able to pass rapidly through the capping and to rise to the level of the outer liquid in less than that time. The capillary opening in the upper end of the tube somewhat retarded the exit of air and, unless the capping was cracked, or slightly perforated, the contained liquid would not reach the level of the outside liquid in five minutes. When the rise was more rapid the apparent reason was recorded. A considerable number of cappings were tested with fresh and used alcohol-formalin solution, and with fresh and used water-formalin solution (Table 5). As will be seen, there was not much difference in the results between the fresh and used solutions. It was clearly indicated, however, that there was a great variation in the rapidity with which the solutions passed through the various cappings.

Twenty cappings were tested in the alcohol-formalin solution. In 5 cases no solution passed through the cappings within the five-minute period; in 6 cases the liquid in the tube rose 1 centimeter or less; in 2 cases it rose between 1 centimeter and 2 centimeters; in 4 cases there was a rise of between 2 centimeters and 3 centimeters; and in 3 cases the liquid rose to the 3 centimeter mark in less than five minutes. Twenty cappings were likewise tested in the water-formalin solution. In 9 cases no solution passed through the cappings in periods varying from 5 to 10 minutes, and, in 1 case, even 60 minutes; in 4 cases there was a rise of liquid in the tube of 1 centimeter or less, one of these after 10 minutes; there were 3 cases of a rise

of between 1 centimeter and 2 centimeters, 1 of these being attained after 5 minutes, 1 after 15, and 1 not until 60 minutes had elapsed; there were 3 cases of a rise of between 2 centimeters and 3 centimeters, 2 of them after 5 minutes, and 1 after 15 minutes had elapsed; in 1 case the liquid rose to the 3-centimeter mark in 3 minutes. When no liquid passed through within the test period, ropy, diseased material was found smeared on the inside of the capping, or the capping appeared unusually thick. As was to be expected, the alcohol solutions passed through more readily in most cases than did water solutions. One capping, not recorded in the table, was submerged in water-formalin solution for three days with no perceptible passage of liquid into the tube.

TABLE 5.—*Tests of the permeability of brood cappings to disinfectants*[1]

Description of cappings	Immersion	Rise in tube
	Minutes	*Centimeters*
Fresh commercial alcohol-formalin solution:		
Dark brown from diseased comb, medium thick	5	2.2
Dark brown from diseased comb, fairly thick	5	.9
Dark brown from diseased comb, quite thin	3	3.0
Dark brown from diseased comb, thick	5	.2
Dark brown from diseased comb, slightly cracked	2	3.0
Dark brown from diseased comb, thick	5	0
Dark brown from diseased comb, medium	5	1.1
Dark brown from diseased comb, thick	5	0
Dark brown from diseased comb, thin	5	2.8
Dark brown from diseased comb, thick	5	0
Used commercial alcohol-formalin solution:		
Light brown from diseased comb, dried, medium thick	5	2.1
Dark brown, old, from diseased comb, dried, thick	5	.9
Brown, old, from diseased comb, medium thin	5	1.2
Scale smeared over inside, thick	5	.1
Dark brown from diseased comb, thick	5	0
Dark brown from diseased comb, thin	3	3.0
Dark brown from diseased comb, some scale in capping	5	[2]0
Dark brown from diseased comb, dry, medium thick	5	2.8
Light brown from diseased comb, good condition	5	1.0
Dark brown from diseased comb, thick	5	.2
Fresh water-formalin solution:		
Dark brown from diseased comb, dried, thick	5	0.9
Dark brown from diseased comb, thick	10	0
Dark brown from diseased comb, thin	15	1.7
Dark brown from diseased comb, normal	5	0
Dark brown from diseased comb, dried, thin	10	0
Do	15	2.4
Dark brown from diseased comb, slightly cracked	5	2.8
Dark brown from diseased comb, thick	10	0
Do	10	0
Do	10	0
Used water-formalin solution:		
Dark brown from diseased comb, dried, thick	10	0.4
Dark brown from diseased comb, dried, cracked	5	1.5
Dark brown from diseased comb, thin	10	0
Dark brown from diseased comb, very thin	5	2.1
Dark brown from diseased comb, thin	60	1.1
Dark brown from diseased comb, small hole	3	3.0
Dark brown from diseased comb, dried, thin	60	0
Dark brown from diseased comb, thin	10	0
Do	5	.7
Dark brown from diseased comb, cracked	5	.8

[1] Tests made at a uniform depth of 3 centimeters below surface of solution. [2] Trace.

To observe what actually takes place in a sealed cell when submerged in a disinfectant solution, some artificial cells were made with pieces of glass tubing of the same diameter as those used in the experiment just described. Pieces of tube about three-quarters of an inch long were sealed at one end and sterilized in a hot-air sterilizer, cotton plugs closing the open ends. Scales containing virulent spores of *Bacillus larvae* removed from diseased combs were then

with aseptic precautions placed in a number of these glass cells, and cappings were sealed on the open ends as in the previous experiment. The sealed glass cells were then submerged for 48 hours in alcohol-formalin and water-formalin solutions, after which they were allowed to dry for a few days. Observations made at the end of 48 hours showed that no perceptible quantity of liquid had entered the cells. Enough moisture had been absorbed through the cappings, however, possibly in the form of vapor or of an indistinguishable film, to cause the dried scales to become slimy or almost ropy, like diseased remains before they have dried down. After drying, cultures were made in the usual manner (Table 6). Three scales out of 20 so treated, 1 in alcohol-formalin solution and 2 in water-formalin solution, apparently were completely sterilized, and from several, most of which had been treated in the alcohol-formalin solution, cultures were made which showed only a comparatively few germinated spores and having a slight growth. This seemed to indicate that not much actual disinfectant gains access to some at least of the sealed cells.

TABLE 6.—*Cultural results of various tests with spores of Bacillus larvae inclosed in artificial glass cells, capped, and treated for 48 hours in formalin solutions*

Solution tested	Cells cultured	Cells showing *B. larvae*	Cells showing no growth	Cells contaminated	Remarks
Fresh alcohol-formalin	5	[1] 5	0	0	Scarcely perceptible growth.
Used alcohol-formalin	5	[2] 3	1	1	Mostly good growth.
Fresh water-formalin	5	[3] 5			Do.
Used water-formalin	5	[3] 3	2	0	Do.
Control, not treated	2	2	0	0	

[1] All showing few spores germinated; one very few.
[2] Two showing many spores germinated.
[3] One showing very few spores germinated.

VACUUM TREATMENT

A method of forcing disinfectant solution into the cells was devised to demonstrate whether alcohol fills all spaces in a submerged comb. Pieces of comb of the same size as those used in previous experiments and containing numerous sealed cells were cut from infected brood combs and submerged in 180 cubic centimeters of disinfectant solution in graduated cylinders. One of these was allowed to soak for 48 hours. The cylinder containing the submerged comb was then subjected to a vacuum of 28 inches, which caused the air still remaining in many of the open cells to rush out in considerable amount and the air in sealed cells to bubble through and in some cases to burst the cappings. When the pressure was allowed to become normal the free liquid in the cylinder had decreased by from 25 to 30 cubic centimeters in displacing the air in the cells. Similar results were obtained by applying the vacuum as soon as the combs were immersed. When combs so treated were subjected to a vacuum again after 48 hours' immersion they were found still to release a few air bubbles from sealed cells. These experiments made it evident that even in open cells of combs immersed for 48 hours at ordinary atmospheric pressure a considerable por-

tion of the cell space is often filled with air. Of course the solution, particularly an alcoholic solution when used with open cells, forms a film on the surface of the cell around the bubble, as has been described by Demuth (*9*), and thus comes in contact with the larval remains. In the case of capped cells, however, under normal atmospheric pressure this bubble can not get out, and only a small quantity of liquid can gain access, in some cases probably not enough to form this moist film.

It was thought at first that this vacuum treatment might be a satisfactory method of disinfecting foulbrood combs, but aside from the cost of apparatus it was found that after this procedure it was practically impossible to remove the disinfectant from the sealed cells, even by means of centrifugal force, particularly from those whose cappings had not been broken, until after removal of the cappings. Liquid in the perfectly capped cells evaporated slowly, and a deposit of solid paraformaldehyde would probably remain in them. This residue has been found objectionable if not positively detrimental to the bees when combs with such a residue are given to a colony. Cultures made from a few combs so treated and allowed to dry for a long time gave completely satisfactory results, no growth being obtained from any cells, either open or sealed. For combs from which all cappings have been completely removed this method of filling all the cells with liquid, thus assuring actual contact of all cell surfaces and cell contents with the water disinfectant, shoud be satisfactory, provided a simple and inexpensive vacuum apparatus can be devised.

PERFORATION OF CAPPINGS

It seemed evident that because of the greater impermeability of many of the cappings the diseased material in some of the sealed cells of the immersed combs was prevented from coming in contact with sufficient disinfectant to kill the spores of *Bacillus larvae* in the infected remains. A preliminary method of perforating brood cappings was tried. By means of a blunt needle holes variable in size and intended to resemble perforations in cappings made by the bees, were made in all cappings, through which the solution might be able to enter the cells more readily. Series of samples of combs with cappings perforated in this manner were treated in both alcohol-formalin and water-formalin solutions, some for 24 and others for 48 hours, as well as in two lots of soap solution for 48 hours. In the 24-hour tests with alcohol-formalin solution, of the 20 scales cultured (Table 8) 5 gave positive growths of *B. larvae*, whereas in the case of the water-formalin solution only 1 scale from a perforated cell, of 20 cultured (Table 10), showed a few germinated spores. In the 48-hour tests with alcohol-formalin solution only 1 scale showed growth out of 20 cultured from perforated cells (Table 7). In the 48-hour tests with water-formalin solution 2 scales, of the 20 cultured from perforated cells, showed a few germinated spores (Table 9). In tests with soap-formalin solution and perforated cappings (Table 1) the contents of all such cells were apparently sterilized as far as this particular experiment was carried. These results indicate that in the case of both solutions the action was aided by perforating the cappings, but in a few instances the sterilizing action

was apparently still incomplete, probably because a trapped air bubble had prevented sufficient solution from passing through the opening.

TABLE 7.—*Cultural results of various tests with samples of comb treated for 48 hours in commercial alcohol-formalin solution*

	Number of consecutive pieces of comb immersed in same lot of solution	Open cells			First comb in series containing open cells showing growth of *B. larvae* in cultures	Sealed cells			First comb in series containing sealed cells showing growth of *B. larvae* in cultures
		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	
Preliminary tests	4	4	0	4	----------	4	[1] 4	0	First.
	3	3	0	3	----------	3	1	2	Second.
	3	3	0	3	----------	3	1	2	First.
	7	7	0	7	----------	7	[1] 2	5	Fifth.
	7	7	0	7	----------	7	1	6	Third.
	8	8	0	8	----------	8	2	6	First.
	5	5	0	5	----------	5	[2] 1	4	Third.
	7	7	0	7	----------	7	5	2	Second.
	7	7	0	7	----------	7	3	4	First.
	10	10	0	10	----------	10	2	8	Ninth.
Open (uncapped) cells compared with sealed cells; used solution.	10	50	0	50	----------	40	[1] 10	30	Third.
	10	50	0	50	----------	40	[3] 18	22	Do.
Open (uncapped) cells compared with sealed cells; fresh solution.	10	50	0	50	----------	50	[4] 25	25	First.
Fresh solution; control for samples washed after treatment.	10	50	0	50	----------	----	----	----	
Open cells, washed in water after treatment	10	50	[2] 3	47	First	----	----	----	
Cells with perforated cappings compared with open cells.	10	10	0	10	----------	10	0	10	
	10	10	0	10	----------	10	1	9	Tenth.

[1] One showed few spores germinated.
[2] Few spores germinated.
[3] Three showed few spores germinated.
[4] Eight showed few spores germinated.

TABLE 8.—*Cultural results of various tests with samples of comb treated for 24 hours in commercial alcohol-formalin solution*

	Number of consecutive pieces of comb immersed in same lot of solution	Open cells			First comb in series containing open cells showing growth of *B. larvae* in cultures	Sealed cells			First comb in series containing sealed cells showing growth of *B. larvae* in cultures
		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	
Preliminary tests	5	5	0	5	----------	5	2	3	First.
Uncapped cells only; used solution	10	50	[1] 2	48	First	----	----	----	
	10	50	0	50	----------	----	----	----	
Uncapped cells only; fresh solution	10	50	[1] 1	49	Tenth	----	----	----	
Fresh solution; control for samples washed after treatment; uncapped cells only.	10	50	[2] 3	47	Sixth	----	----	----	
Open cells, washed in water after treatment	10	50	[2] 4	46	First	----	----	----	
Cells with perforated cappings compared with open cells.	10	10	0	10	----------	10	3	7	Eighth.
	10	10	0	10	----------	10	[2] 2	8	Fourth.

[1] Few spores germinated. [2] One showed few spores germinated. [3] Two showed few spores germinated.

TABLE 9.—*Cultural results of various tests with samples of comb treated for 48 hours in water-formalin solution*

	Number of consecutive pieces of comb immersed in same lot of solution	Open cells				Sealed cells			
		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	First comb in series containing open cells showing growth of *B. larvae* in cultures	Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	First comb in series containing sealed cells showing growth of *B. larvae* in cultures
Preliminary tests	6	6	0	6		6	[1] 4	2	Third.
	6	6	0	6		6	[2] 3	3	Do.
	6	6	0	6		6	1	5	Fifth.
Open (uncapped) cells compared with sealed cells; used solution.	10	50	0	50		40	[1] 12	28	Fourth.
	10	50	0	50		40	[2] 10	30	Do.
Open (uncapped) cells compared with sealed cells; fresh solution.	10	50	0	50		50	[3] 15	35	Third.
Fresh solution; control for samples washed after treatment.	10	50	0	50					
Open cells, washed in water after treatment	10	50	0	50					
Cells with perforated cappings compared with open cells.	10	10	0	10		10	[1] 2	8	Fourth
	10	10	0	10		10	0	10	

[1] Two showed few spores germinated.
[2] One showed few spores germinated.
[3] Seven showed few spores germinated.

TABLE 10.—*Cultural results of various tests with samples of comb treated for 24 hours in water-formalin solution*

	Number of consecutive pieces of comb immersed in same lot of solution	Open cells				Sealed cells			
		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	First comb in series containing open cells showing growth of *B. larvae* in cultures	Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	First comb in series containing sealed cells showing growth of *B. larvae* in cultures
Open cells only; used solution	10	50	0	50					
	10	50	0	50					
Open cells only; fresh solution	10	50	0	50					
Fresh solution; control for samples washed after treatment.	10	50	0	50					
Open cells washed in water after treatment	10	50	[1] 3	47	First				
Cells with perforated cappings compared with open cells.	10	10	0	10		10	[2] 1	9	Seventh.
	10	10	0	10		10	0	10	

[1] Germination of few spores; doubtful in the case of two cultures.
[2] Showed few spores germinated.

CAPPINGS REMOVED WITH KNIFE

Considerable experimenting with various methods of opening cappings sufficiently to allow easy access of solutions into the cells demonstrated that the only satisfactory way is to use an uncapping knife and actually to cut away the cappings, as is done when uncapping honey, before the combs are placed in the disinfectant solution. If the knife is sharp and well heated, brood cells may be as easily uncapped as honey cells, leaving a sharp, complete opening and adding but little to the labor factor in handling the combs. If the knife is

dull, it will tear the comb, closing many of the cells, so that the solution can not enter. All honey cells that previously have not been extracted can be uncapped at the same time as the brood cells.[2]

Several series of combs uncapped in this manner were treated with both the alcohol-formalin and water-formalin solutions for 24 and for 48 hours. In some of the 48-hour tests the cappings were cut from only one side of the combs, so that cultures for comparison might be made from sealed cells at the same time and to obtain definite figures for the percentage of sterilization of the sealed cells. To reduce the probable error arising from the missing of occasional cells that would give growth, 5 open cells and 5 sealed cells from each comb, instead of only 1, were cultured. In the 24-hour tests and in the last 48-hour series only open cells were cultured, since from the previous experiments it was found that 24 hours and in many cases even 48 hours was not invariably sufficient to sterilize all sealed cells. In tabulating these results the number of combs in each series of samples and the total number of cells cultured is recorded.

As indicated in Table 7, a total of 281 scales from open cells of combs treated for 48 hours in alcohol-formalin solution, including the cells originally open and those opened by uncapping but excluding those washed after treatment, were cultured, none of which gave any growth of *Bacillus larvae*. A total of 191 scales from 191 sealed cells of the same combs (omitting 20 combs having perforations in the "sealed" cells and other combs from which cultures were made from open cells only) gave 115 cultures showing no growth of *B. larvae*, 61 showing good growth, and 15 showing a few germinated spores, or 75 positive cultures; 39.8 per cent of these cells were therefore not sterilized. In the 24-hour tests of scales from open cells, including all considered in Table 8 except those washed after treatment, a total of 225 scales cultured gave 219 tubes showing no growth of *B. larvae*, 2 showing good growth, and 4 showing a few germinated spores, or 6 positive cultures, a percentage of 2.7 for open cells not sterilized. Only one short (preliminary) series of scales from sealed cells of combs treated for 24 hours was tested, with 2 out of 5 cultures showing growth of *B. larvae*.

As indicated in Table 9, a total of 238 scales from open cells from samples of combs treated for 48 hours in water-formalin solution, including cells originally open and cells uncapped, but no cells washed after treatment, were cultured, none of which gave a growth of *Bacillus larvae*. A total of 148 scales from sealed cells from the same combs when cultured gave 103 showing no growth of *B. larvae*, 32 showing good growth, and 13 showing a few germinated spores, or 45 positive cultures, a percentage of 30.4 for sealed cells not sterilized. In the 24-hour tests of scales from similar open cells cultured (Table 10), 220 scales were cultured, all the cultures showing no growth of *B. larvae*. No 24-hour sealed cells were tested with

[2] In a recent article Vincens (*24*), who has charge of the station for apicultural research of the Institute of Agricultural Research at Cagnes, France, describes his method for removing cappings and soaking the contents of the cells previous to immersion in a water-formalin solution. A jet of water reduced to a spray is shot against the surface of the comb at an angle with considerable force. This destroys or perforates the cappings and fills the cells. After 12 hours the combs are again treated in like manner. Then after passage through an extractor the combs, which are now thoroughly wet, are immersed in a water-formalin solution containing only 10 per cent formalin for 24 hours, after which the solution is removed and the combs allowed to dry. Vincens reports success so far with this method used on a small scale.

water-formalin solution. A shorter period of immersion than 24 hours was not tried during these investigations.

WASHING COMBS

In one or two instances combs have been treated with alcohol-formalin solution in cold weather and allowed to dry slowly at a comparatively low temperature. These, when returned to the bees, have caused considerable trouble, and even loss of colonies by desertion of the bees. Because of the retarded rate of evaporation at lower temperatures, the formaldehyde, instead of evaporating, undergoes a transformation to the solid and much less volatile paraformaldehyde. This is probably more often true of the water-formalin solution, which evaporates more slowly. When evaporation takes place slowly, a residue sufficient to be poisonous or obnoxious to bees may remain in the cells for a considerable time. Washing combs in water after treatment was tried by Jones (*15*) to remove the odor of formaldehyde, and has been recommended by Corkins (*7*) as a preventive against this difficulty. No observations in the apiary or results of cultural tests after such treatment have been recorded. Several series of combs were tested, using pieces from which the cappings had been cut, treating some for 24 and some for 48 hours, and comparing the combs allowed to dry without washing with those washed in water.

As indicated in Table 7, 50 scales were cultured from open cells of combs washed in water after treatment for 48 hours in alcohol-formalin solution. Of these, 3 cultures showed only a few germinated spores of *Bacillus larvae*. The control series of combs with open cells (Table 7) not washed in water gave no cultures showing any growth. In the 24-hour tests 50 scales were cultured from open cells of combs washed in water after treatment for 24 hours in alcohol-formalin solution (Table 8). Of these, 2 cultures showed good growth of *B. larvae* and 2 a few germinated spores. The control series of combs (Table 8) not washed gave 2 cultures showing good growth, and 1 showing a few germinated spores.

As shown in Table 9, 50 scales were cultured from open cells of combs washed in water after treatment for 48 hours in water-formalin solution. None of the cultures gave any growth of *Bacillus larvae*. The control series of combs (Table 9) that were not washed gave from open cells no cultures showing any growth. In the 24-hour tests 50 scales were cultured from open cells of combs washed in water after treatment for 24 hours in water-formalin solution (Table 10); of these, 1 culture showed good growth of *B. larvae* and 2 showed a few doubtful germinated spores. The control series of combs with open cells (Table 10) not washed in water gave no cultures showing any growth of *B. larvae*.

DISCUSSION

Throughout these preliminary laboratory experiments on the germicidal efficiency of the various types of disinfectants tested, no solution was found which sterilized the contents of all sealed cells uniformly in all of the several series of combs immersed in it. This fact is probably of importance in the sterilization of brood

combs infected with American foulbrood by means of various formaldehyde disinfectant solutions as used by beekeepers. It was found to be true even with solutions of low surface tension and supposedly good penetration, such as alcohol-formalin solution, as well as with solutions having high wetting and spreading powers with regard to waxy surfaces, such as soap-formalin solutions. In all of the several series of combs treated, even after a treatment of 48 hours, cultures from varying numbers of scales from capped cells gave growths of *Bacillus larvae*. A comparison of the percentage of sealed cells not sterilized indicates that the efficiency of the alcohol-formalin solution is relatively no greater in this regard than that of water-formalin solution.

The explanation of this failure of the formaldehyde disinfectants to sterilize all the sealed brood may be found in the varying permeability of the brood cappings, particularly many of those covering remains of American foulbrood. In some cases no solution was able to pass through the cappings, low surface tension of the liquid and solvent action on the wax seeming to be ineffective factors. In these cases the glue-like remains of diseased dead larvæ have often become smeared over the inner surface of the cappings during the routine handling of the combs, drying there and clogging the pores of the cappings. As a result, no solution could penetrate them, at least within a length of time practicable for treating combs without subjecting them to action causing undesirable deterioration. Even on the same comb there is wide variation in the structure of the cappings themselves. As can be noted in the various tables, there is great irregularity as to which comb of a series shows the first sealed cells giving a growth of *Bacillus larvae*, indicating incomplete sterilization. The experiments with glass cells indicate the possibility that the complete sterilization achieved in many of the sealed cells is brought about by the penetration into the cells of formaldehyde gas and water vapor liberated from the disinfectant solution, rather than by actual liquid sufficient to cause sterilization. Experiments with the use of a vacuum in treating combs indicated that relatively little solution enters the majority of sealed cells at usual atmospheric pressure, but that the method should be effective with all uncapped cells. These facts may explain the few cases of recurrence of disease so far noted with the method now in use, and serve as the basis of a prediction of more cases in the future. Why more such recurrences have not been observed is not easy to explain, but it is to be remembered that this method of disinfecting combs has been in use for only about two years and much concerning it is yet to be learned.

A certain proportion of the cultures of scales from sealed cells (25 per cent of those treated with alcohol-formalin solution and about 33 per cent of those treated with water-formalin solution) showed comparatively few germinated spores. In some of these cases no growth was visible on the surface of the culture medium. Some few of these cultures on subculturing failed to show further growth or germination. The question here arises whether contact with formaldehyde insufficient to kill may lower the vitality of these organisms so that they fail to continue growth, or may lower their virulence, thereby inhibiting infection of healthy colonies from such sources. It is also possible that an insufficient number of spores

remain alive to start the disease, as experience in cultivating *Bacillus larvae* from diseased material on artificial culture media has demonstrated that a certain quantity of inoculum is necessary to start growth, whereas growth will not begin when there are only a few spores. These are among the problems awaiting solution.

Another important fact brought out by the various tests of solutions containing 20 per cent of formalin is that in the case of all combs that were treated for 48 hours no cultures made from scales taken from open cells or from cells the cappings of which had been removed gave any growth of *Bacillus larvae*. For this length of treatment complete sterilization was accomplished by the water-formalin solution as well as by the alcohol-formalin solution. In the case of the 24-hour tests a few of the scales from open cells of combs treated with alcohol-formalin solution failed to be completely sterilized, 6 of the 220 such scales cultured giving growths of *B. larvae*. Apparently a 24-hour treatment is somewhat below the minimum time in which complete sterilization of the scales in open cells may be expected. On the other hand, none of the cultures from 220 scales from open cells of comb treated 24 hours in water-formalin solution showed any growth of *B. larvae*. These results indicate that the water-formalin solution is more efficient as a germicide for American foulbrood than is the alcohol-formalin solution, provided the solutions come in actual contact with the scales for at least 24 hours.

All cappings, those over brood cells as well as honey cappings, should be carefully removed to insure sterilization of combs infected with American foulbrood. Even when this is done, apparently a 48-hour treatment is still necessary when an alcohol-formalin solution is used. From the fact that all cultures were negative which were made from scales in open cells of combs treated 24 hours in a water solution containing 20 per cent of formalin, a treatment of 24 hours in such a solution appears to be sufficient. When, after combs have been soaked in the disinfectant solution, the excess liquid has been removed in an extractor, and the combs are allowed to dry without further treatment, there is still some disinfectant left which continues to act while they are drying, until it is entirely evaporated. If circumstances, such as the necessity for drying treated combs at a comparatively low temperature, require the washing of the treated combs in water in order to prevent the formation of an undesirable residue caused by the retarded evaporation, the combs should be treated 48 hours, whether in water-formalin or in alcohol-formalin solution; an additional 12 hours in the case of alcohol-formalin solution would give a greater margin of safety. If this is not done, the discontinuance of the germicidal action may permit a few scales to emerge with spores still capable of causing disease, while a period of treatment shorter than 48 hours before washing in water would be entirely insufficient for complete germicidal action.

The uncapping of all brood cells removes the necessity of using a solution capable of penetrating the wax. With the cappings removed, the dissolving action, or penetration of the wax of the comb, seems of questionable value. It is noteworthy that not one case has definitely been recorded of disease resulting from the use in healthy colonies of tons of comb foundation that for years has been made from wax rendered from combs once containing American foulbrood.

The uncapping of all brood cells eliminates the necessity for a solution with low surface tension. The much less expensive water-formalin solution will be found to enter the open cells sufficiently to soften, loosen, and sterilize the scales as effectively as an alcoholic solution, although the entrance may not be quite so rapid. The slower entrance into the cells is more than counterbalanced by the greater germicidal efficiency of formalin in the presence of water as compared with that of the same disinfectant in an alcoholic solution. The water-formalin solution readily softens and penetrates the masses of pollen in cells, since stored pollen is held together by small amounts of honey, readily soluble in water. Aside from the countless spores contained in the diseased remains and those in the infected honey or pollen, practically the only other spores that might in any way get to healthy larvæ and cause disease are not in masses, but are scattered individually over the surfaces of the comb, having accidentally been carried there by the bees in their work.

White (*27, p. 32*) has shown that spores suspended in a 20 per cent formalin solution would be killed inside of a few hours; it follows that when individual spores on combs come in direct contact with the disinfectant they must be killed in a comparatively short time. If, as was found to be the case in the laboratory experiments, soaking for 24 hours of scales of American foulbrood in open cells kills all spores embedded in them, probably immersion for only a few hours is necessary to kill these spores on the comb surfaces, as would be the case with dry extracting combs that have been in diseased colonies but have contained no dead brood. The few spores that might become embedded in propolis are as negligible as those in the wax.

The uncapping of all brood cells, as well as of any sealed cells of honey in diseased brood combs, naturally adds somewhat to the labor cost of treating these combs; but if results in the apiary compare at all with the results in the laboratory, the lowering of the cost by elimination of the alcohol, using only formalin in water, will more than offset the slight additional labor cost. However, before treatment it is almost always necessary to uncap some sealed honey in combs from diseased colonies; if the brood is uncapped at the same time, comparatively little extra effort or time will be required. The washing of combs, when necessary after treatment for 48 hours in the water-formalin solution, also adds to the labor costs; but in this case again the extra labor should be repaid by the results.

The data presented indicate that a 20 per cent solution of formalin in water is the most efficient as well as the most economical disinfectant so far found for the sterilization of combs infected with American foulbrood, provided the cappings are all completely removed. It is hoped that apiary tests will be made to determine the practicability of these results. Naturally, even with the most reliable process, carelessness in handling the combs, and more particularly carelessness in the treatment of the diseased colonies from which the combs are taken, will be fatal to success in gaining control over this disease which has thus far caused such great losses. The must approved methods for the treatment of American foulbrood have been discussed elsewhere.[3]

[3] Methods of treatment and control of American foulbrood are discussed in United States Department of Agriculture Farmers' Bulletin 1084.

CONCLUSIONS

A number of soap solutions were found unsatisfactory as carriers for 20 per cent of their volume of formalin to be used as a disinfectant for treating American foulbrood combs; (1) because of the difficulty of controlling the reaction of the solution and of preventing the precipitation of the soap and (2) because of the failure of the soap solution to penetrate all cappings of sealed cells sufficiently to kill all spores of *Bacillus larvae* contained in the diseased material therein, and to do this within a period of 48 hours.

Certain liquids of low surface tension other than ethyl alcohol, such as acetone and iso-propyl alcohol, are somewhat more efficient as carriers for formalin than most of the solutions tried, including ethyl alcohol, as indicated by the comparatively few sealed cells which failed to be completely sterilized by them within a period of 48 hours. On the other hand, these liquids are too expensive for practical use, even if satisfactory as carriers for the formalin. Less expensive liquids tried, such as a commercial methyl-ethyl ketone, are unsatisfactory because of failure to sterilize scales in sealed cells in 48 hours.

Miscellaneous disinfectants such as dilute hydrochloric acid, various dilutions of iodine, acetic acid added to water-formalin solution to increase its penetrating power, and such substances as gelatine, added to water-formalin solution to increase the wetting and spreading powers, are all unsatisfactory as disinfectants for sterilizing American foulbrood combs, since none of them completely sterilizes all sealed cells in 48 hours. The hydrochloric acid solutions and all but quite concentrated iodine solutions even fail to sterilize all the open cells. Formaldehyde, used as formalin solution, when employed in sufficient proportions is the most efficient and practical germicidal agent thus far used for the purpose of disinfecting American foulbrood combs. A solution containing less than 20 per cent of formalin is found to be unreliable.

The results obtained with various dilutions of alcohol and of alcohol-formalin solution as the carrier for 20 per cent of their volume of formalin are not sufficiently complete to warrant conclusions as to their relative efficiency. All of these solutions are unsatisfactory, since they do not completely sterilize all sealed cells in 48 hours. A 20 per cent solution of formalin in water, without alcohol, is slightly less efficient than the alcoholic solutions in sterilizing in 48 hours the contents of sealed cells, because of its failure to penetrate many of the cappings; but it sterilizes all open cells in that period.

The commercial alcohol-formalin solution, like all the other solutions tested, fails to sterilize completely the scales from all sealed cells with a treatment of 48 hours.

The variation in the permeability of many of the brood-cell cappings accounts for the failure to sterilize many of the sealed cells within a period of 48 hours. In some cases cappings completely resist the passage of liquid or vapors into the cells, thereby making low surface tension and solvent action unavailing within the period of time practicable for the satisfactory treatment of combs.

The results of experiments with glass observation cells, as well as of the application of a vacuum to the solutions containing combs

under treatment, indicate that little if any actual liquid enters cells where the capping is intact and more or less impermeable. It is probable that when such sealed cells are sterilized the disinfection is brought about by the passage through the capping of gas and vapor liberated from the solution.

The perforation of brood cappings adds to the efficiency of the disinfectant solutions, both alcoholic and water; but, aside from the difficulty of doing this in a practical way, the sterilization of such cells is not always uniformly complete, even after a treatment of 48 hours.

To obtain uniformly complete sterilization of all infected cells in American foulbrood combs, no matter what solution is used, all cappings, covering both brood and honey, should be carefully removed before the combs are immersed in the solution. This can be easily done if care is used, with a hot, sharp, uncapping knife, and adds little to the labor costs.

The sterilization of combs infected with American foulbrood, when all the cells are uncapped and alcohol-formalin solution is used, requires, if complete from the standpoint of the cultural results obtained, more than a 24-hour treatment. A treatment of 48 hours would give a margin of safety.

The sterilization of combs infected with American foulbrood, when all the cells are uncapped and a 20 per cent solution of formalin in water is used, if complete from the standpoint of the cultural results obtained, requires treatment for 24 hours at least. A somewhat longer period would give a greater margin of safety.

The washing of combs in water, to prevent the formation of a residue of paraformaldehyde after treatment with disinfectants containing 20 per cent of formalin, should not be attempted unless the combs have been treated at least 48 hours in the solution. A somewhat longer period would give a greater margin of safety, particularly with the alcohol-formalin solution.

To reduce the chance of missing any cells that have not been completely sterilized, when testing the efficiency of any disinfectant solution, a sufficiently large number of cells should be cultured. The proportion should be approximately from 3 to 5 per cent of the cells of each class, open and closed, so distributed as to represent fairly all the cells treated.

In view of the cultural results obtained, a 20 per cent solution of formalin in water was found the most satisfactory disinfectant for use in sterilizing combs infected with American foulbrood, with regard to both germicidal action and low cost, provided the proper precautions are taken. All honey should be extracted, all brood cappings should be completely removed, and the combs should be treated at least 24 hours, or 48 hours if it is found desirable to wash them in water after treatment. Before such a procedure can be recommended unreservedly exhaustive tests must be carried out under apiary conditions.

Care must be taken not only with the process of disinfecting combs infected with American foulbrood, no matter what solution or method is used, but equal or greater care must be exercised in the treatment of the diseased colonies themselves to eliminate the danger of recurrence of disease from that source. The successful sterilization of the combs will otherwise be of little avail.

LITERATURE CITED

(1) BORCHERT, A.
1921. Die Formaldehyddesinfection in der Bienenwirtschaft in der Form des Autanverfahrens sowie experimentelle Untersuchungen über die Tiefenwirkung des mit Wasserdampf gesättigten Formaldehydgases. *In* Arb. Biol. Reichsanstalt Land- u. Forstw., vol. 10, Heft 6, pp. 522–557.

(2) BYER, J. L.
1924. Alcohol-formalin treatment. *In* Gleanings Bee Cult., vol. 52, No. 4, pp. 230–231.

(3) ———
1924. Disease in treated combs. *In* Gleanings Bee Cult., vol. 52, No. 9, pp. 584–585.

(4) ———
1924. In Ontario. [Further report on recurrence of disease with treated combs.] *In* Gleanings Bee Cult., vol. 52, No. 10, p. 662.

(5) ———
1925. In Ontario. [Note on alcohol-formalin vs. water-formalin treatment.] *In* Gleanings Bee Cult., vol. 53, No. 1, pp. 38–39.

(6) COOPER, W. F., and NUTTALL, W. H.
1915. The theory of wetting, and the determination of the wetting power of dipping and spraying fluids containing a soap basis. *In* Jour. Agr. Sci., vol. 7, pt. 2, pp. 219–239, 3 figs.

(7) CORKINS, C. L.
1924. In Wyoming. [Note on washing of combs with water after treatment with alcohol-formalin solution; to prevent losses from formation of paraformaldehyde.] *In* Gleanings Bee Cult., vol. 52, No. 10, p. 659.

(8) DEMUTH, G. S.
1923. American foulbrood ousted. *In* Gleanings Bee Cult., vol. 51, No. 11, pp. 712–717.

(9) ———
1924. Sterilizing diseased combs. *In* Gleanings Bee Cult., vol. 52, No 4, p. 211.

(10) GRIFFIN, R. C.
1920. The solubility of metals in acids containing formaldehyde. *In* Jour. Indus. and Engin. Chem., vol. 12, No. 12, pp. 1159–1160.

(11) HUTZELMAN, J. C.
1922. Can the combs be saved? New treatment for American foulbrood by immersion in disinfectant solution. *In* Gleanings Bee Cult., vol. 50, No. 12, pp. 764–766.

(12) ———
1924. Honeycomb sterilizer. [U. S. Patent No. 1511762. Patented Oct. 14, 1924.] U. S. Patent Office, Off. Gaz., vol. 327, p. 387.

(13) JARVIS, G. L.
1925. Apiary experience in treating combs. *In* Beekeeper, vol. 33, No. 3, pp. 27, 38.

(14) JONES, DAN H.
1924. Control of American foulbrood. Summary of some laboratory tests with disinfectants in this disease. *In* Gleanings Bee Cult., vol. 52, No. 6, pp. 364–365 and 396.

(15) ———
1925. Chemical treatment of foulbrood. *In* Beekeeper, vol. 33, No. 1, pp. 4–5 and 10.

(16) KING, GEORGE E.
1925. Disinfecting diseased combs. *In* Gleanings Bee Cult., vol. 53, No. 7, pp. 438–440.

(17) KRÖNIG, B., and PAUL, TH.
1897. Die chemischen Grundlagen der Lehre von der Giftwirkung und Desinfection. *In* Ztschr. Hyg. u. Infectionskrank., vol. 25, pp. 1–112.

(18) MAASSEN, A., and BORCHERT, A.
1920. Über die Bekämpfung der antsteckenden Bienenkrankheiten und über Entseuchungsversuche mit Formaldehyd in der Form des Autanverfahrens. *In* Mitt. Biol. Reichsanstalt Land- u. Forstw., Heft 18, pp. 151–156.

(19) MOORE, WILLIAM.
1921. Spreading and adherence of arsenical sprays. Univ. Minn. Agr. Exp. Sta. Tech. Bul. 2, 50 pp.

(20) RIDEAL, S., and RIDEAL, E. K.
1921. Chemical disinfection and sterilization. London. 313 pp.

(21) STURTEVANT, A. P.
1924. The development of American foulbrood in relation to the metabolism of its causative organism. *In* Jour. Agr. Research, vol. 28, No. 2, pp. 129–168.

(22) UNITED STATES TREASURY DEPARTMENT, BUREAU OF INTERNAL REVENUE.
1920. Regulation No. 61. Laws and regulations relative to the production, tax payment, etc., of industrial alcohol and to the manufacture, sale, and use of denatured alcohol. 107 pp.

(23) VANSELL, G. H.
1925. Promising experiments in sterilizing foulbrood combs. *In* Amer. Bee Jour., vol. 65, No. 2, p. 55.

(24) VINCENS, F.
1925. Désinfection des rayons contaminées par la "loque pernicieuse" à l'aide d'une solution aqueuse de formol. *In* La France Apic., vol. 31, No. 8, pp. 174–175.

(25) WHITE, G. F.
1904. The further investigation of the diseases affecting the apiaries in the State of New York. *In* N. Y. State Dept. Agr. Ann. Rpt. Comr. 1903, pp. 103–114.

(26) ———
1906. The bacteria of the apiary, with special reference to bee diseases. U. S. Dept. Agr. Bur. Ent., Tech. Ser. 14, 50 pp.

(27) ———
1920. American foulbrood. U. S. Dept. Agr. Bul. 809.

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29

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